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# Zoonotic potential of the parasites of the Khuman Wadi: environmental and health repercussions in Moulay Idriss Zerhoun, Morocco

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**Abstract.** The Oued Khoumane river, which flows through the city of Moulay Idriss Zerhoun, receives discharges of raw wastewater, posing a risk to public health and the environment. To assess the extent of intestinal parasitosis, 72 water samples were collected monthly in 2022 from six sites.Analyses, conducted using a concentration technique (WHO method) for helminth eggs and MIF staining for protozoan cysts, revealed significant and diverse parasitic contamination. A total of 17 parasite genera were identified, with a predominance of protozoa (50.5%), followed by nematodes (29.8%), cestodes (16.2%), and trematodes (3.5%). This study highlights an alarming parasitic load, of both human and animal origin, which poses a direct threat to the health of populations using this water and degrades the aquatic ecosystem. It underscores the urgent need to implement preventive and corrective measures.

**Keywords**: Khoumane Wadi, wastewater, parasites, potential, environment, health

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# 1 Introduction

Growing pressures from demographic, economic and environmental imperatives are driving increased demand for water resources, putting considerable pressure on surface waters, leading many countries to consider wastewater as a strategic resource in its own right [1]. These resources are nevertheless largely exposed to contamination by industrial, domestic and agricultural discharges, with well-documented health risks when this water is reused without adequate treatment for irrigation [2]. In Morocco, where water shortages are structural, untreated reuse of wastewater for agricultural irrigation is a common practice, especially in urban areas where treatment infrastructure remains limited: only 7 of the 60 largest cities have wastewater treatment plants [3], which makes agricultural reuse particularly problematic from a health point of view [1]. This practice exposes nearly 8,000 hectares of agricultural land, mainly dedicated to fodder, fruit, cereal and vegetable crops, to proven pathogenic contamination, with major implications for public health, animal health and the integrity of aquatic ecosystems, as shown by several studies on the impact of wastewater irrigation on food crops.

In the city of Moulay Idris Zerhoun, which is our study area, 39 farms located upstream and downstream of the direct discharge points of raw wastewater, covering an area of 350.8 hectares, use this water without prior treatment, thus reproducing conditions already described in other urban and peri-urban contexts in developing countries [1]. Among emerging contaminants, helminths are identified as one of the most critical parasitic threats due to their low infectious dose, prolonged environmental resistance, and ability to persist in untreated irrigation systems, consistent with recent syntheses on sewage- and sludge-associated helminthiasis.

The objective of this study is to measure the parasite load in the waters of the Oued Khoumane and to assess the impact of this on the environment and public health, with particular reference to the helminth reduction values proposed for recycled water intended for agriculture. Secondly, the study will aim to quantify the health risks associated with irrigation with untreated water, based on the risk management guidelines related to wastewater reuse in agriculture. It will also guide integrated water resources management policies in rural and urban areas, integrating controlled wastewater recovery approaches while limiting the transmission of helminths and other pathogens [1].

# 2. Materials and Methods

## 2.1 Study environment

The town of Moulay Idriss Zerhoun, a spiritual and tourist site housing the mausoleum of Idriss I, founder of the Idrisside dynasty, is located 25 km from Meknes. Its territory is crossed by the Oued Khoumane, a tributary of the Oued R'dom. This wadi, fed by several sources, the main one being the Ain Shench source, also acts as a collector and outlet for the municipal domestic wastewater. This situation creates a health and environmental risk of concern for local populations. The economy of the Moulay Idriss Zerhoun region is mainly structured around the agricultural sector. This activity, mostly rainfed, is characterized by an annual monoculture system. Production is dominated by legume and cereal crops, as well as arboriculture (olive, caper and carob trees) whose vulnerability to the degradation of natural resources was analysed by El Amrani-Paaza et al. [4]. In parallel, extensive breeding, mainly sheep and cattle, is practiced on a limited scale. Together, this primary sector is the main provider of subsistence for a significant part of the working population. The local economic ecosystem is complemented by two periodic commercial infrastructures: an operational slaughterhouse every Thursday and a weekly souk held on Saturdays, which ensures the distribution of a wide range of goods and commodities. Indeed, the polluted waters of the wadi are used for livestock watering, agricultural irrigation, swimming and various other activities, practices that join the general problems of irrigation with wastewater. Contamination of the groundwater by infiltration is also to be feared, with significant risks to human health and the quality of groundwater resources. As part of a twelve-month study in 2022, six sampling stations were selected along an upstream-downstream gradient of the Oued Khoumane. Among these, station S2 corresponds precisely to the direct discharge point of a main wastewater collector in the municipality, thus constituting a site of major interest for the assessment of pollution.

## 2.2 Methodology

A monthly sampling campaign was conducted over a period of twelve months, from January to December 20, 22, along the Oued Khoumane, at the municipality of Moulay Idriss Zerhoun (Morocco). A device of six sampling stations (S1 to S6) was established according to an upstream-downstream longitudinal gradient **(Fig. 1)**. This spatial configuration was chosen to characterize the variability of anthropogenic pressures exerted on the watercourse.

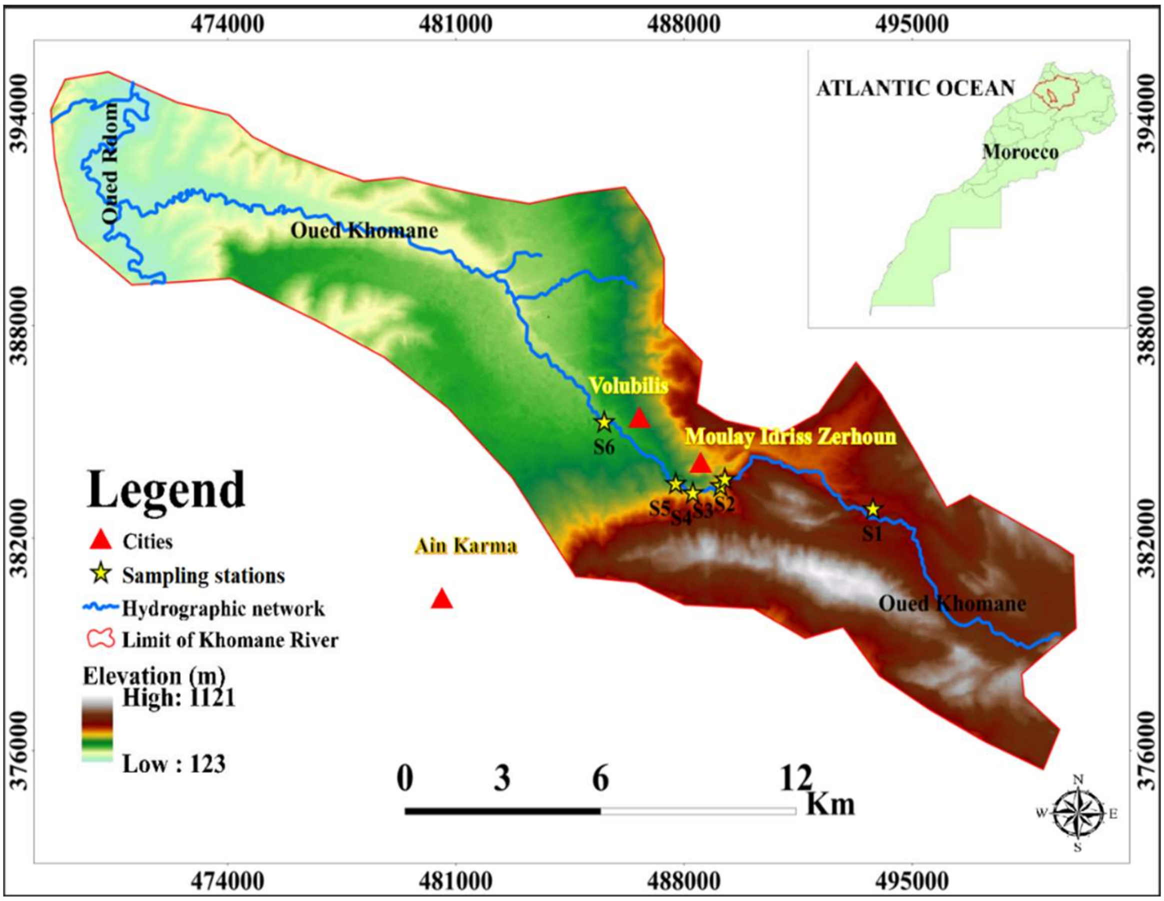


**Fig. 1** : Location of the Study Area and Sampling Stations

Parasitological analysis was performed using Arther Fizerald Fox's flotation concentration technique. In addition to its safety and moderate cost (using sucrose as a reagent), this method was chosen for its performance and reliability in the isolation of nematode eggs, including *Ascaris sp.* and *Trichuris sp.,* reported by who as serious pathogens.
The quantification of helminth eggs and protozoan cysts is performed through the use of a MacMaster slide, which is examined under the illumination of a light microscope. This methodology is consistent with the counting and standardisation protocols employed for the

assessment of parasite loads in epidemiological and environmental research, particularly in the context of the analysis of fecal sludge.

The results of the parasitological analysis are expressed using the formula proposed by WHO [5]. To determine the total number of helminth eggs and protozoan cysts per litre (N), this specific formula is applied, making it possible to link the number of parasitic elements counted to the initial volume of the sample, in the same methodological logic as that used to link microbiological or parasitic contamination of water and excreta to health risks in developing regions.

$$\mathbf{N = A.\ X / P.\ V}$$

With:

**N** = number of parasites per liter of wastewater sample.
**A** = number of parasites counted on the Mac Master blade
**X** = volume of the final product (ml).
**P** = capacity of the Mac Master blade (0.3 ml).
**V** = volume of the initial wastewater sample to be analyzed (2 liters)

In order to study the variability between stations, the data collected in this study were analyzed on the XLstat 2014 software by the ANOVA test. A subsequent Tukey HSD test was used in cases where the analysis of variance revealed significant differences. The statistical analysis of the data was carried out using XLstat 2014 software. In order to assess spatial variability between sampling stations, an Analysis of Variance (ANOVA) was applied to the calculated ecological indicators, namely abundance, and pest load (expressed in units per litre). The normal distribution of the residues was previously verified by the Shapiro-Wilk test. In cases where the ANOVA indicated significant differences, a post-hoc analysis via the Tukey HSD (Honestly Significant Difference) test was conducted to identify specific station pairs with significant deviations. In addition, an analysis (AFC) was carried out for the spatio-temporal visualization of parasitic loads was developed to illustrate their dynamics

## 3 Results and discussion

### 3.1 Qualitative Results: Specific Wealth

The analysis of the samples, collected monthly at each station for twelve months, made it possible to identify four major parasite taxa: Nematodes, Cestodes, Trematodes and Protozoa. These groups, represented by a total of 17 distinct genders. (**Tables 1 and 2**).

**Table 1**: Classification of helminths identified during the year 2022

| Helminth eggs | Subclass | Family | Gender |
|---|---|---|---|
| *Trichocephalus trichiura* | Nematodes | Trichuridae | *Trichuris* |
| *Ascaris lumbricoides* | Nematodes | Ascarididae | *Ascaris* |
| *Ascaris vermicularis* | Nematodes | Oxyuridae | *Enterobius* |
| *Ancylostoma duodenale* | Nematodes | Ancylostomatidae | *Ancylostoma* |
| *Capillaria sp.* | Nematodes | Capillariidae | *Capillaria* |
| *Ascaris canis* | Nematodes | Toxocaridae | *Toxocara* |

| | | | |
|---|---|---|---|
| *Strongyloides intestinalis* | Nematodes | Strongyloididae | *Strongyloides* |
| *Beef Tapeworm* | Cestodes | Taeniidae | *Taenia* |
| *Dwarf tapeworm* | Cestodes | Hymenolepidae | *Hymenolepis* |
| *Hymenolepis diminuta* | Cestodes | Hymenolepidae | *Hymenolepis* |
| *Common liver fluke* | Trematodes. | Fasciolidae | *Fasciola* |
| *Schistosoma sp.* | Trematodes. | Schistosomatidae | *Schistosoma* |
| *Trichostrongylus sp.* | Nematodes | Trichostrongylidae | *Trichostrongylus* |

**Table 2:** Classification of protozoa identified during the year 2022

| **Protozoan cysts** | **Class** | **Family** | **Gender** |
|---|---|---|---|
| *Balantidium coli* | Heterotrichea | Balantidiidae | *Balantidion* |
| *Entamoeba histolytica* | Lobosea | Entamoebidae | *Entameba* |
| *Entamoeba coli* | Lobosea | Entamoebidae | *Entameba* |
| *Giardia lamblia* | Trepomonadea | Giardiidae | *Giardia* |

## 3.2 Quantitative results

### 3.2.1 *Percentage of positive samples*

Analysis of the samples revealed a significant prevalence of different intestinal parasites, with a clear breakdown into three distinct groups suggesting clear stratification within the samples studied. Parasites with a high prevalence (>80%) are dominated by *Entamoeba coli* (88.9%), *Giardia lamblia* (87.5%) and *Entamoeba histolytica* (83.3%), rates comparable to those reported by Kiros et al. [6] in contexts of major fecal contamination. This high prevalence indicates major fecal contamination of the environment.

An intermediate group (50-80%) is mainly composed of helminths such as *Ascaris*, *Enterovirus*, *Blastocystis* and *Taenia*, indicating active fecal-oral transmission. Finally, less frequent parasites (<50%) such as *Fasciola hepatica*, *Trichostrongylus* and *Schistosoma* are present in limited quantities, suggesting more specific modes of transmission or restricted geographical distribution.This parasitic distribution reveals a situation of polyparasitism characteristic of poor sanitary conditions, with a lack of access to drinking water and sanitation, requiring urgent interventions in terms of collective hygiene, health education and systematic deworming programs to improve the public health of the population studied

### 3.2.2 *Abundance*

Our results show a significant predominance of protozoa (Fig. 2), which account for more than half of the parasites detected (50.5%), suggesting fecal-mediated transmission of water and food contamination, as well as resistance in the form of cysts in the environment. Nematodes are the second largest group (29.8%), reflecting the prevalence of fecally orally transmitted intestinal helminthiasis in areas with poor hygiene. Cestodes account for 16.2% of parasites, indicating possible consumption of undercooked meat or exposure to intermediate hosts. As for trematodes, they account for only 3.5%, probably due to more complex life cycles requiring specific intermediate hosts (molluscs) and therefore a more restricted geographical distribution. This parasitic distribution, dominated by protozoa and nematodes (80.3% cumulative), is characteristic of regions where sanitary conditions are precarious, with limited access to drinking water, poor sanitation and inadequate hygiene practices. These results underscore the urgency of implementing integrated prevention programmes combining improved health infrastructure, hygiene education and mass pest management.

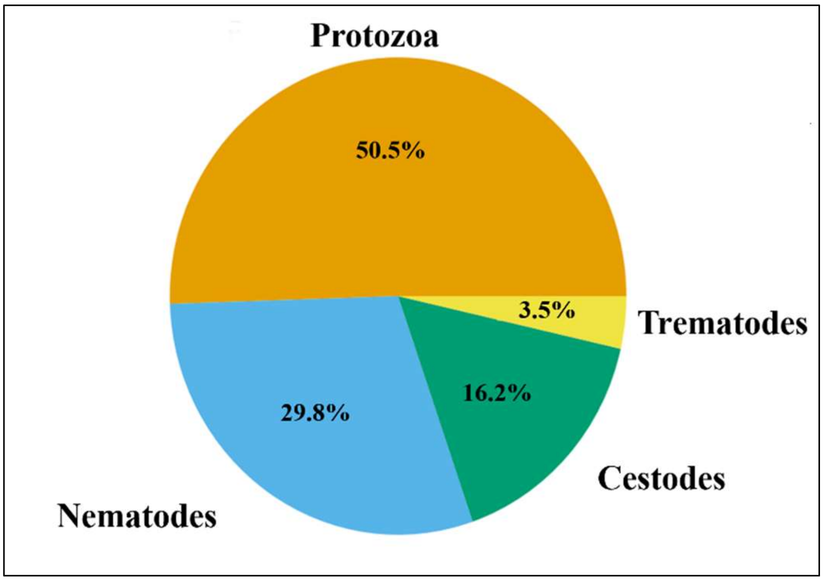
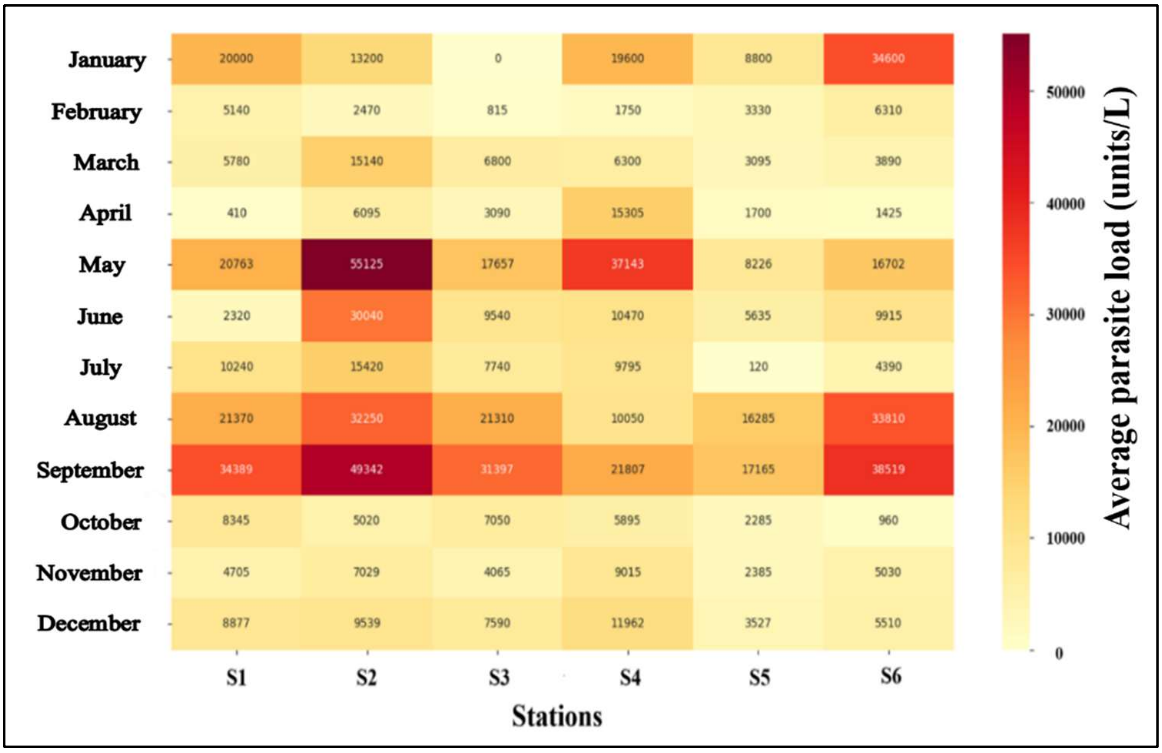


**Fig. 2**: Prevalence of parasite eggs/cysts

### *3.2.3 Monthly spatial and temporal evolution*

Analysis of the spatio-temporal variation in mean parasitic load (in units/L) across six sampling stations (S1 to S6) reveals the existence of distinct seasonal and geographical patterns (Fig. 3). The marked intensification of parasitic contamination during the hot and humid months, particularly in May and September, is particularly notable, with peaks reaching 55,125 units/L in S2 station in May and 49,342 units/L in the same station in September. These observations suggest a correlation with climatic conditions favorable to parasite survival and transmission (high temperatures, precipitation). Station S2 stands out as the most critical point, with parasitic loads consistently high throughout the year, probably indicating a source of major contamination (sewage discharge, densely populated area). Station S5, on the other hand, generally has the lowest loads, probably due to its greater distance from sources of contamination. The months of January, May, August and September show widespread contamination across all stations, with values often exceeding 20,000 units/L, while the periods of February, April, October and November record relatively more moderate parasitic loads. This heterogeneous distribution highlights the combined influence of environmental (rainy season promoting runoff and parasite dispersal), anthropogenic and geographical (proximity to sources of contamination) factors, highlighting the need for continuous monitoring of water quality, targeted interventions on hot spots identified as S2, and reinforced preventive measures during high-risk periods to protect public health, consistent with the socio-economic impacts analyzed by El Amrani-Paaza et al. [4] and the spatio-temporal epidemiology of Soulaymani et al. [7].



| | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|
| January | 20000 | 13200 | 0 | 19600 | 8800 | 34600 |
| February | 5140 | 2470 | 815 | 1750 | 3330 | 6310 |
| March | 5780 | 15140 | 6800 | 6300 | 3095 | 3890 |
| April | 410 | 6095 | 3090 | 15305 | 1700 | 1425 |
| May | 20763 | 55125 | 17657 | 37143 | 8226 | 16702 |
| June | 2320 | 30040 | 9540 | 10470 | 5635 | 9915 |
| July | 10240 | 15420 | 7740 | 9795 | 120 | 4390 |
| August | 21370 | 32250 | 21310 | 10050 | 16285 | 33810 |
| September | 34389 | 49342 | 31397 | 21807 | 17165 | 38519 |
| October | 8345 | 5020 | 7050 | 5895 | 2285 | 960 |
| November | 4705 | 7029 | 4065 | 9015 | 2385 | 5030 |
| December | 8877 | 9539 | 7590 | 11962 | 3527 | 5510 |

**Fig. 3**: Prevalence of parasite eggs/cysts

### *3.2.4 Statistical approach and ecological interpretation of parasitic charges*

To present a complete analysis, from data collection to ecological and health interpretation, including statistical tests, while correcting apparent inconsistencies (in particular between two sets of Shapiro-Wilk test results).

The evaluation of the distribution of parasitic loads was first carried out using the Shapiro-Wilk test. The results (**Table** 3) showed that the four classes of parasites (Protozoa, Nematodes, Cestodes and Trematodes) had distributions compatible with normality ($p > 0.05$ for all classes). This normality of distributions suggests a homogeneous and progressive contamination along the watercourse, without a dominant isolated pollution point. The data can therefore be considered sufficiently normal to justify the use of a parametric test.

**Table 3:** Shapiro-Wilk Test Results

| Class | Statistics W | p-value | Interpretation |
|---|---|---|---|
| Protozoa | 9,558 | 0,7865 | Normal distribution (not rejected) |
| Nematodes | 0,8455 | 1447 | Normal distribution (not rejected) |
| Cestodes | 0.9531 | 7656 | Normal distribution (not rejected) |
| Trematodes | 0,8652 | 0.2078 | Normal distribution (not rejected) |

Based on the Shapiro-Wilk test results, a one-factor ANOVA was used to compare parasitic loads between the six stations. Analyses (**Table 4)** revealed highly significant differences ($p<0.001$) for all parasite classes. Tukey's post-hoc tests made it possible to identify more precisely the contrasts between stations. In particular, increasing contamination is observed downstream, with station S6 having significantly higher protozoan loads than upstream stations S1 and S5. Stations S2 and S4, subject to urban and agricultural discharges respectively, also show levels of contamination of concern.

**Table 4 :** ANOVA Test Results

| Class | p-value (ANOVA) | Significant Differences |
|---|---|---|
| Protozoa | < 0.001 | S6 > S1, S5; S2, S4 > S5 |
| Nematodes | < 0.001 | S6, S4 > S1, S5 |
| Cestodes | < 0.001 | S2, S3 > S5 |
| Trematodes | < 0.001 | S5 > S1 (risk of bilharzia) |

From an ecological and health point of view, these results highlight several worrying trends. Station S5, in particular, is distinguished by a marked presence of trematodes of the genus *Schistosoma*, indicating a potential risk of bilharzia for exposed populations, as in the spatio-temporal analyses described by Li et al. [8]. In addition, the persistence of cestodes in stations S2 and S3 indicates chronic fecal contamination of the environment.

Factorial Correspondence Analysis (FCA) applied to parasitological data makes it possible to reveal synthetically and visually the underlying structures of parasite distribution according to sampling sites and seasonal variations, this highlightings the non-obvious associations between parasite profiles and specific ecological or anthropogenic conditions, according to the multivariate approaches of Mewamba et al. [9].

The combined AFC (stations + months) makes it possible to identify significant associations between certain sites and specific parasites, reflecting differentiated contamination dynamics. In biplots (Fig. 4), the first two dimensions explain 70.1% and 29.9% of the total variance, showing a clear separation between stations: S1 and S5 are associated with *Trichuris trichiura* and *Ascaris lumbricoides* in the upper right quadrant, S2 shows a strong correlation with *Schistosoma sp* and *Trichostrongylus sp* in the upper left quadrant, while S3, S4 and S6 cluster in the right with *Fasciola hepatica*, *Entamoeba histolytica* and *Giardia lamblia*, suggesting distinct parasite profiles according to geographical areas probably related to local sources of contamination and specific environmental conditions, consistent with the distribution models of Ayob et al. [10].

Factorial analysis reveals a complex spatio-temporal structure of parasite distribution across the six stations, confirming observations of spatio-temporal variation in parasite load and ACP biplots: station S6 is distinguished by a marked association with *Giardia lamblia* and *Trichostrongylus sp,* particularly during the summer months (August-September as observed in the heat map with high values), suggesting livestock-related zootechnical contamination. The S1, S2 and S4 stations have profiles dominated by faeco-oral parasites (*Entamoeba coli*, *Balantidium coli*) and systematically high parasitic loads, in particular S2 which reaches 55,125 units/L in May and maintains critical values throughout the warm period, indicating major sanitation failures.The spatial clustering observed in the PCA between S2, S3 and S4 is explained by their similar parasitic profile including *Hymenolepis nana*, *Taenia saginata* and various protozoa, associated with risky feeding practices or contact with intermediate hosts, while S5, having the lowest loads in the temporal analysis, nevertheless maintains a specific presence of *Fasciola hepatica* and *Schistosoma sp*, parasites requiring intermediate molluscs and indicating localized marshy areas. The PCA-confirmed seasonal dimension of the months shows a net summer upsurge (May-September) of soil pests like *Strongyloides stercoralis* and *Ascaris lumbricoides*, favored by the high humidity and temperatures visible in Figure 3 with peaks exceeding 30,000-50,000 units/L, contrasting with a winter decline where only directly transmitted pests like *Enterobius vermicularis* persist, reflecting a climate-independent continuous domestic circulation, with the combined influence of genetic and environmental factors, in the contexts of hyperendemicity and coinfections observed by Mas-Coma et al. [11].This multivariate analysis demonstrates that parasitic contamination follows patterns structured by the interaction between environmental (climate, hydrology), anthropogenic (sanitation, population density) and zootechnical (livestock, agricultural practices) factors, making it possible to guide targeted interventions: reinforced veterinary control at S6, urgent improvement of sanitation at S2 identified as a critical point, preventive interventions before the rainy season in all stations, and health education campaigns adapted to the specific parasitic profiles of each site.

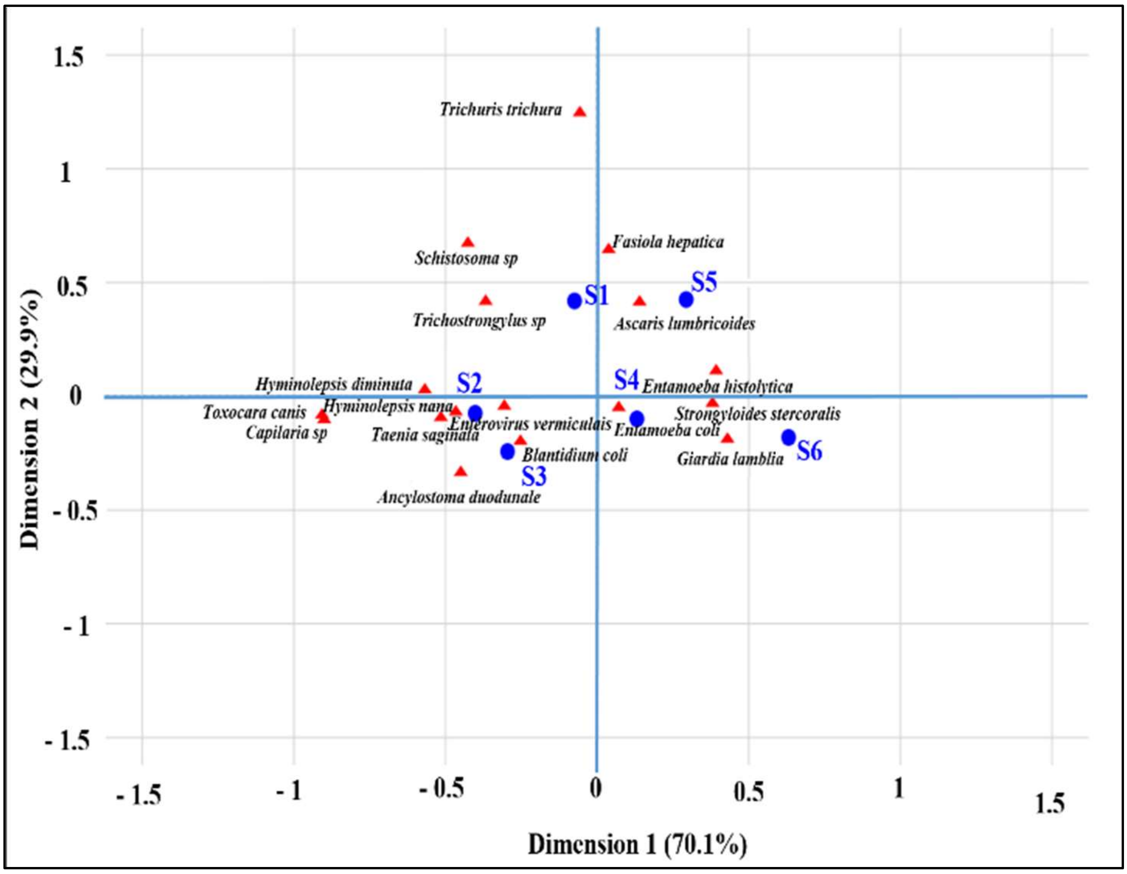


**Fig. 4**: Relationship between stations and parasites

In biplots (Fig. 5), with dimensions explaining 66.2% and 38.8% of the variance, reveal a marked seasonal structure: the cold months (October, November, December) in the lower right quadrant are associated with *Strongyloides stercoralis*, the transition months (March, April, May) in the central part show parasitic diversity including *Balantidium coli*, *Entamoeba coli* and *Hymenolepis diminuta*, while January and February in the upper right quadrant are correlated with *Trichuris trichiura* and *Ascaris lumbricoides*, and September in the left part shows particular associations with other parasites, as in the global *Strongyloides* distributions described by Fleitas et al . [12].

This multivariate analysis demonstrates that parasite distribution is influenced simultaneously by geographical factors (proximity to sources of contamination, hydrological characteristics of the stations) and temporal factors (seasonal variations in temperature, precipitation, agricultural practices), with some species such as *Trichuris* and *Ascaris* showing greater spatial and temporal ubiquity while others such as *Schistosoma* have more restricted distributions, crucial information for developing targeted monitoring and control strategies adapted to the specific parasite profiles of each station and time of year, following the principal component analysis approaches. Seasonally (Fig. 5), the PCA confirms the dynamics observed in the spatio-temporal variation of parasite load, with a parasitic upsurge during the warm months (May to September), when conditions of high temperature and humidity favor the survival and transmission of soil parasites (*Strongyloides stercoralis*) and protozoa (*Giardia lamblia, Balantidium coli*), while the cold months (October to January) show a general decline in parasitic activity with limited persistence of certain agents such as *Enterobius vermicularis*.

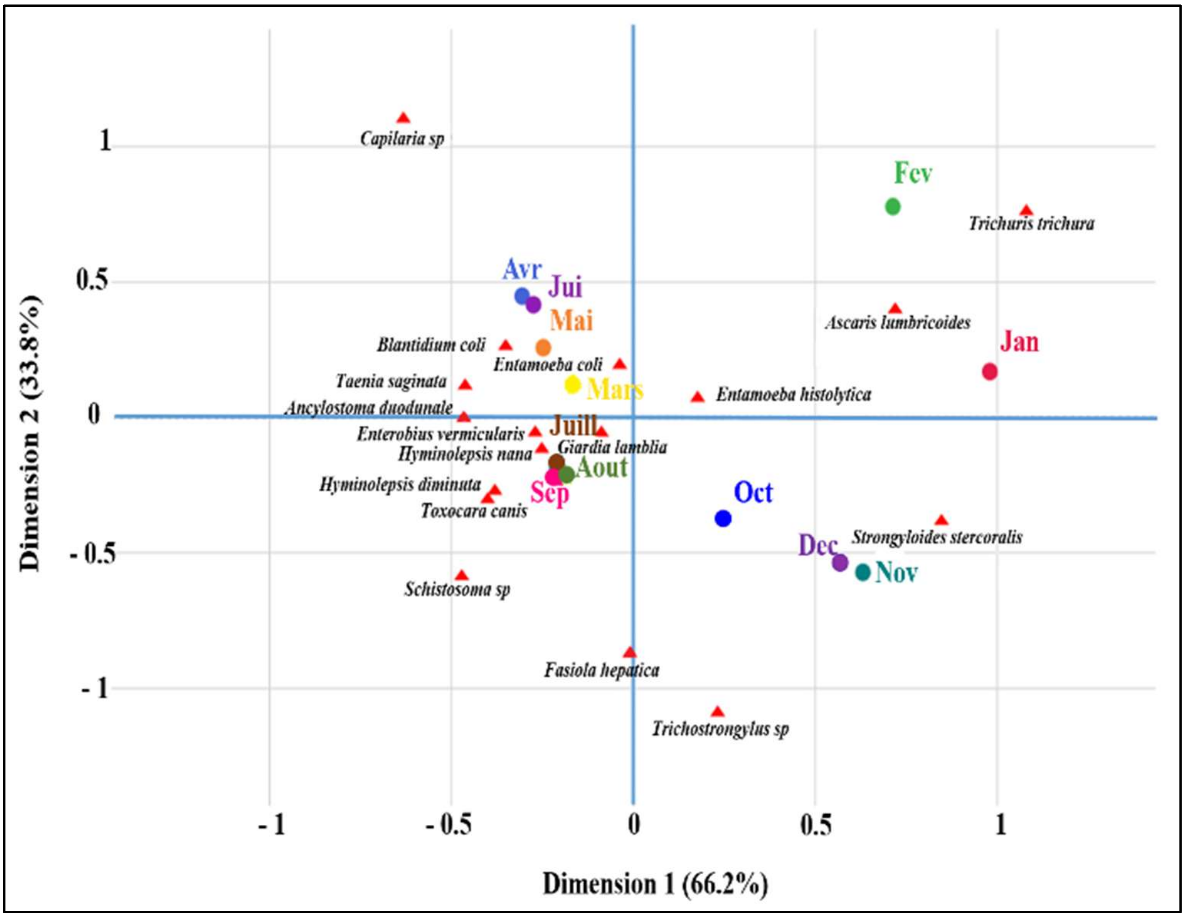


**Fig. 5**: Seasonal dynamics of pests

Cross-referencing the ACP analyses presented above with parasite distribution data, we observe that some stations such as S6 and S1 are distinguished by a strong association with *Giardia lamblia, Ascaris lumbricoides* and *Trichuris trichiura*, suggesting fecal contamination related to untreated effluents, as reported by Aliyo et al. [13]. While station S2, identified as a critical point in the heat map, shows high loads of various protozoa (*Balantidium coli, Entamoeba coli*) and helminths, indicating major sanitation problems, as described in the factors associated with parasitic infections by Firdu et al . [14]. Station S5, with the lowest parasitic loads in the temporal analysis, nevertheless maintains a presence of specific parasites such as *Fasciola hepatica*, probably related to particular environmental conditions favouring intermediate molluscs.

# 4. Conclusion

Our comprehensive study reveals widespread and alarming environmental parasitic contamination, characterized by ecological complexity and a major health risk. The results converge to establish an unequivocal observation: the watercourse constitutes a reservoir and an active vector of intestinal parasites of human and animal origin, reflecting serious failures in the management of wastewater and excreta, as well as precarious sanitary conditions for the surrounding populations. Of particular concern is parasitic prevalence and diversity. The detection of 17 different parasite genera, with a net dominance of protozoa (50.5%) – including *Entamoeba coli*, *Giardia lamblia* and *Entamoeba histolytica* at prevalences exceeding (80%), followed by nematodes (29.8%), attests to massive and continuous fecal contamination. This distribution, characteristic of polyparasitism in poor sanitation, signals active and widespread fecal-oral transmission.

The spatio-temporal analysis highlighted the existence of marked seasonal patterns, with parasite load peaks reaching more than 55,000 units/L during the hot and humid months (May, September), corroborates the determining influence of climatic factors (temperature, precipitation) on the survival and dispersion of pathogens in the environment. Spatially, the identification of critical points, in particular station S2 subjected to urban discharges, demonstrates the direct impact of localized anthropogenic pressures. The gradation of contamination along the watercourse confirms a phenomenon of accumulation downstream, although each station has a distinct parasitological profile related to its specific sources of contamination (urban, agricultural, zoonotic).

From a health and ecologicalpoint of view, the implications are serious. The persistence of such a parasitic load in a watercourse used for domestic and agricultural purposes represents an ongoing threat to public health, exposing populations to a wide spectrum of gastrointestinal and systemic pathologies, with an increased risk for vulnerable groups. At the ecosystem level, this organic and biological pollution contributes to the degradation of the quality of the aquatic environment and disrupts its balance.

The data imperatively call for the urgent and integrated implementation of corrective and preventive measures: prioritization of interventions on identified hot spots (such as S2); drastic strengthening of sanitation and water treatment infrastructures; targeted campaigns of massive deworming and health education adapted to local profiles; and the establishment of a continuous epidemiological and environmental surveillance system, integrating the spatio-temporal dimension highlighted.

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**Data availability statement**: The datasets analyzed during the current study are available from the corresponding author on reasonable request. All the data are included in the present study

**Author contribution statement:** All authors reviewed the manuscript.

Abdelhak Saidi: writing, original draft, Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Moulay Lafdil Belghiti: Formal analysis and data curation, Youssef Ouballouk: Visualization, Brahim Elhilali: Visualization, Mohcine Sadki: review, Lhoussine Jait: review, Abdelkhalek Belkhiri: Methodology, Youssef Haddadi: review & editing, Driss Bengoumi: Preparation, Iman Taha: Visualization, Abdelkader Chahlaoui: Supervision.

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